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Award Number: DAMFinaD17-03-1-0575

TITLE: Proteomic Analyses of Nipple Fluids for Early Detection of Breast Cancer

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REPORT DATE: October 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
1-OCT-2007	Final	15 SEP 2003 - 14 SEP 2007

4. TITLE AND SUBTITLE

Proteomic Analyses of Nipple Fluids for Early Detection of Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-03-1-0575

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Dana-Farber Cancer Institute
Boston, MA 02115

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Our purpose is to combine the well-established method of breast nipple fluid collection with new proteomics methodology for analyses of complex protein mixtures, in order to seek a better test for early breast cancer. The scope of our work is far-reaching, as our results could have a significant impact on the ability to detect occult breast cancers at earlier stages than is possible with current cancer detection methods. To date, we have completed a pilot test of 12 patients to optimize collection and lab storage and analysis conditions. Since the previous year, our numbers have increased progressively: we collected 196 nipple aspirate fluid (NAF) samples (168 from case subjects and 29 from control subjects). Our modification of the collection protocol to collect NAF directly with capillary has been successful. This collection procedure is more efficient providing more useable samples for lab analysis. We have run test samples using SELDI-TOF methodology that showed significant numbers of peaks on IMAC40 chips thereby validating both the collection and analysis protocols. In summary, we have developed a workable protocol for nipple fluid collection that produces consistent quantitative protein analysis. We continue to focus our efforts on sample collection.

15. SUBJECT TERMS
early breast cancer detection; screening; proteomics; breast nipple fluid; women at high risk

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		6	USAMRMC
					19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

When breast cancers are diagnosed by self-examination, clinical examination and/or mammography, the tumor is usually comprised of millions of genetically unstable cancer cells, including subclones differing in responses to therapy. The goal of our project is to find a new method to detect occult breast cancers at earlier stages than is possible with current cancer detection methods. To achieve our goals, we have combined the well-established method of breast nipple fluid collection with the new proteomics methodology for analyses of complex protein mixtures, in order to seek a better test for early breast cancer. We aim to collect breast nipple fluids from 200 women with yet-untreated breast cancer (cases), and 200 cancer-free controls and perform proteomic analyses in 100 cases and 100 controls to identify nipple fluid protein patterns associated with the presence of breast cancer. Specific proteins and patterns will be validated by repeating the proteomic studies on a second blinded series of fluids from another series of 100 cases and 100 controls, link laboratory results with clinical and diagnostic data, and score the sensitivity and specificity of the assay. If successful, the proteomics assay might permit earlier breast cancer diagnosis, and thereby reduce disease morbidity and mortality.

BODY:

Task 1. Subject recruitment and consent (years 1 and 2)

a. We modified our existing IRB-approved protocol (93-020: Collection and Laboratory Study of Breast Nipple Fluid Using a Nipple Suction Cup) to include proteomic analyses among the laboratory assays, renaming it 03-249. After receiving full IRB and DOD approval of 03-249, we have closed 93-020 for recruitment, leaving it open only for data analysis. There is no longer a need to collect under 93-020.
b. We continue to obtain consent from eligible subjects and their physicians to collect breast nipple fluid samples and demographic and clinical data from 200 women with newly diagnosed breast cancer. The limiting factor to enrollment of cases has been the availability of eligible patients. In an effort to increase enrollment of cases, our current focus lies on the expansion of opportunities for case recruitment within the DOD-approved sites. We are currently screening the patients of six surgeons and approaching eligible cases on the day of their breast surgery. This new recruitment strategy has resulted in a recent increase in case accrual, and an 80 percent consent rate of the women approached for the study. We are actively looking to add more surgeons to the study in an effort to increase the pool of eligible cases.
c. Additionally, we continue to consent 200 cancer-free control subjects examined at the mammography centers and breast clinics within Dana-Farber/Harvard Cancer Center. Enrollment of control subjects continues at a steady rate.

Task 2. Nipple fluid collection (years 1 and 2)

a. We continue to use our previously developed methods to collect nipple fluids into capillary tubes from subjects. We have collected 196 NAF samples to date. Of these, 168 are samples from control subjects, and 27 are from case subjects.
b. Nipple aspirate fluid samples are now being extracted by surgeons in the operating room prior to surgery. This new method of collection has yielded us an average rate of two cases collected per week.
c. Samples are being delivered to Dr Miron's laboratory where they are stored at –140 degrees C after recording the volume.

Task 3. Biomarker discovery in nipple fluid (years 1 and 2)

a. We have optimized lab conditions to analyze NAF after collection. Optimization includes: (1) having established a NAF recovery protocol from the capillary tubes using a denaturing buffer

and centrifugation, which is well suited for high-throughput studies; and (2) running test samples with the SELDI-TOF methodology. Significant numbers of peaks were detected in those samples on IMAC40 chips thereby validating both the collection and analysis protocols.

b. Further analysis will be performed on a complete set of aspirates at the end of the collection phase of the study. We aim to perform proteomic analyses on 100 aliquots of nipple fluids from patients with breast cancer and 100 cancer-free women, using SELDI-TOF methodology using each of the chip surfaces in duplicate. *(No work has been done on this portion pending completion of the collection; such work can only be done in batches to minimize other sources of variation)*

Task 4. Identification and validation of nipple fluid biomarkers for cancer (years 2 and 3)

(To date, no work has been done on this task)

a. Identify protein patterns in the arrays that differentiate nipple fluids obtained from cancerous breasts as compared with tumor-free breasts.
b. Catalogue the protein markers present in nipple fluids from cancerous breasts that are absent from nipple fluids of cancer-free women.
c. To validate the preliminary findings from the first set of samples, a third party will code the second series of 100 nipple fluid samples from breast cancer patients and 100 samples from cancer-free women.
d. Repeat the SELDI-TOF analyses in a blinded manner on the 100 case samples and 100 controls.
e. Analyze data for the 200 blinded samples and score the source of the sample as a cancer-bearing breast, normal breast or indeterminate origin.
f. Break the sample codes and quantify the sensitivity and specificity of the assay in differentiating breast cancer cases from healthy controls.
g. Identify causes of false-positive and false-negatives.

Task 5. Dissemination of results and, if appropriate, organize multi-center studies (year 3)

(To date, no work has been done on this task)

a. Prepare manuscript(s) for peer-review and publication.
b. If the proteomic analysis is shown to have high predictive value, develop a multi-center collaborative to re-confirm the results in a larger study.
c. Seek additional funding to further refine the assay, and submit a Traditional Research Proposal.

KEY RESEARCH ACCOMPLISHMENTS:

- We have worked out a collection method that is efficient and obtains a high-yield of nipple aspirate fluid.
- We have determined that resuspending the aspirate in a small amount of PBS allows for full recovery of the sample.
- We have developed systematic protocol for collection that is easily adaptable to other sites.

REPORTABLE OUTCOMES:

None to date

CONCLUSION:

To date, we have developed a workable protocol for nipple aspirate collection that produces consistent quantitative protein analysis. We have completed a pilot collection phase to optimize collection and analysis methods. We have successfully collected 196 NAF samples. We have developed a successful method of collecting nipple fluid samples and are averaging two cases

collected per week. We continue to develop new strategies for recruitment and look forward to completing continuing sample collection and beginning analysis.

REFERENCES:
None

APPENDICES:
None